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Behavioral Toxicity of Trihalomethane Contaminants of Drinking Water in Mice

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16. ABSTRACT

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Behavioral Toxicity of Trihalomethane Contaminants of Drinking Water in Mice

by Robert L. Balster* and Joseph F. Borzelleca*

The behavioral toxicity of trichloromethane (TCM), dichlorobromomethane (DCBM), dibromochloromethane (DBCM) and tribromomethane (TBM) was evaluated following oral administration in mice. A variety of dosage regimens and behavioral measures were used. Studies included acute dose effect, 14- and 90-day treatments at 300 and 3000 times the estimated average human daily intake of contaminated drinking water, 30 days of 100 mg/kg/day, and 60 days of 100 and 400 mg/kg/day. In addition, TCM was tested for the production of taste aversions with 10-day administration and for behavioral teratology in offspring following extensive perinatal exposure. The ED_{50} for acute effects on a screen test of motor performance was about 500 mg/kg for all four trihalomethanes. The 14-day treatments had no effect on swimming behavior and the 90-day treatments had no effect on bar clinging, a test of motor coordination, and a measure of exploratory behavior. None of the compounds produced effects on passive-avoidance learning following 100 mg/kg/day for 30 days. TCM, DBCM and TBM elicited clear effects at both 100 and 400 mg/kg/day on operant behavior when administered for 60 days. DBCM elicited clear effects at 400 mg/kg/day. These effects on operant behavior were seen following the first dose and tolerance tended to develop. Thus, there was no evidence from these studies for a progressive neurotoxicity from trihalomethanes in adult mice. A behavioral teratology study was also conducted with TCM. Both parents were treated with 31.1 mg/kg/day TCM, and treatment of the dam continued throughout gestation and lactation. No clear evidence for behavioral effects in the offspring were observed. The most sensitive measure for the effects of TCM was the taste aversion paradigm in which saccharin aversions were produced after a single treatment of 30 mg/kg.

Trihalomethanes are present in finished drinking water in relatively large amounts due to their formation during water disinfection by chlorination (*1–3*). As part of a larger project to assess the toxicity of drinking water contaminants in mice, a number of studies of the behavioral effects of four trihalomethanes were conducted. This paper describes our early approach to behavioral toxicity testing and reviews the results.

Several considerations led to the design of the experiments to be reported here. The mouse was chosen as the experimental subject largely due to its planned use by other components of the overall project. The mouse is not commonly used in behavioral toxicology hence most of the tests were developed or adapted specifically for this project. Concern with drinking water exposure dictated that the studies use an oral route of administration and that the studies include long term, daily, low dose exposures. Since the behavioral toxicity of these types of exposures to trihalomethanes had not been evaluated, there was no basis for predicting which behavioral functions would be most likely affected by these contaminants. For this reason, a battery of behavioral tests were used and many different exposure conditions were evaluated. Finally, some of the dosage levels were chosen based upon the highest levels reported in finished water supplies at the time this research was initiated (*1*). Most of the doses for long-term exposure studies were chosen to represent 300 and 3000 times the estimated average daily human intake (ADI) assuming 2 liters per day intake of these highly contaminated supplies.

Methods

Subjects

With the exception of the behavioral teratology and taste aversion experiments, all studies were

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carried out with adult male ICR mice (Flow Laboratories, Dublin, Va.). They arrived in the weight range of 19–24 g and were initially housed in groups of eight to ten in standard laboratory mouse cages equipped with automatic watering using tap water. They were allowed at least one week of adaptation before any experimentation was carried out. With the exception of food deprivation for operant studies, mice were allowed continuous access to Ralston-Purina Rodent Laboratory Chow no. 5001. No special precautions were taken to use contaminant-free water since we were interested in studying large doses of selected contaminants administered by gavage. Consumption of Richmond tap water was considered to be a more natural background against which the effects of the trihalomethanes could be studied.

After the adaptation period, subjects were assigned randomly to groups. For most studies this resulted in cages of six to eight mice receiving identical treatments. For taste aversion and operant studies the subjects were individually housed. For the teratology studies both female and male ICR mice were used, and the behavioral studies were carried out on the offspring. For the taste aversion study, adult male mice of the CD-1 strain (Charles River, Wilmington, Mass.) were used.

Behavioral Tests

A number of different behavioral tests were used to assess the effects of both acute and repeated trihalomethane administration. With the exception of the operant studies, all testing was carried out by an experimenter blind as to the dosing conditions. The following is a description of the behavioral tests.

Screen Test. This is a simple test of motor performance described originally by Coughenour et al. (4) and is similar in some respects to the more common rotorod. Mice were placed individually on top of a horizontal wire mesh screen 13 × 13 cm square placed 31 cm above the table top. Six such screens were mounted in a row on a metal rod which, when rotated, turned the screens upside down. The number of mice which climbed to the top of the screen by 15, 30 and 60 sec was counted. This measure was used to study the acute effects for each of the trihalomethanes as well as the motor performance of mice receiving repeated doses. For the latter, treatment groups were compared to control groups by chi-square analysis. Two tests were conducted 30 min apart beginning 24 hr after the last dose, and the results at 15, 30 and 60 sec for each test were analyzed separately.

Cling Test. Mice were tested for their ability to cling to a small diameter bar suspended horizontally 29 cm above a table surface. Latency to fall was recorded up to a maximum of 60 sec. Treatment and control groups were compared by one-way analysis of variance.

Hole-Board. The hole-board has been used by a number of investigators (*5,6*) to assess exploratory activity. The apparatus consisted of a 43-cm square plastic box with a false floor positioned 17 cm above the bottom. The bottom was divided into four sections, and two of the sections contained a complex object consisting of hardware (nuts, bolts and wires) attached to a wooden block, and two were empty. A 3-cm diameter hole in the false floor was situated directly above each of the four compartments. Mice were placed individually on the false floor and the number of head dips into holes above compartments with or without objects were recorded separately. Head dips were scored by an observer when the subjects' nose and eyes were inserted into the hole. The apparatus was wiped clean after the testing of each subject. The test period was 3 min and subjects were tested 24 hr after the last of repeated doses of the trihalomethanes. Treatment and control groups were compared by an unweighted means two-way analysis of variance with repeated measures on one factor (groups × objects present or absent).

Swimming Endurance. Swimming behavior was assessed in a 30 × 30 cm cylindrical tank containing water at 15°C. Animals were made heavier by taping a length of lead wire equal to 10% of the subjects' body weight onto their back. The weight was placed in a way not to interfere with leg movement. A line was drawn 2 cm below the water level and the latency to sink below the line was recorded. The animals were then immediately removed from the tank. No subjects drowned in carrying out these studies. Animals were tested individually 24 hr after the last dose of repeated dosing. Results for treatment and control groups were compared by one-way analysis of variance.

Passive-Avoidance Learning. One trial passive shock avoidance was assessed following repeated contaminant exposure. A trapezoidal-shaped box was divided into a clear "safe" compartment and a dark "shock" compartment by a guillotine door. On a training trial a subject was placed in the safe compartment and the latency to enter the dark compartment was recorded. When the subject entered the dark compartment the door was lowered and the subject was given 5 sec of unavoidable 40-V ac shock via grid plates comprising the floor of the shock compartment. One hour and 24 hr later, test trials were conducted. They were identical to the training trial except shock was omitted. Training trials were conducted 24 hr following the last repeated dose of the trihalomethanes. The latencies for treat-

ment and vehicle control groups were compared by two-way analysis of variance with repeated measures on one factor (groups × test period).

Operant Behavior. Studies of the effects of repeated administration of trihalomethanes on schedule-controlled performance were conducted in operant chambers designed for this study. The apparatus and general procedures for care and feeding of subjects, lever press training, and experimental control and data collection have been described previously (*7*). Briefly, individually housed mice were given 2 hr per day restricted access to food following daily 30-min experimental sessions. They were trained to lever press for dipper presentations of sweetened milk on a differential reinforcement of low rate 10 sec (DRL 10) schedule, i.e., only interresponse times of 10 sec or greater were reinforced. Subjects were trained over a period of about two months until response rates were stable. Subjects were then habituated to daily vehicle gavage (5 ml/kg Emulphor:water 1:8) given 30 min prior to the onset of the session. At this point daily treatment was initiated. The subjects in each treatment group (N = 6 to 13) received a daily intubation 30 min prior to each session, seven days per week. On occasional days when sessions were not conducted, treatments were continued as usual. Daily treatments were given for 60 days or until the subjects died, which commonly occurred in high dose groups. After 60 days the treatments were discontinued and the subjects were tested for an additional 3 days following vehicle gavage. The primary measures of behavior were response rates and reinforcement rates. These measures for the last pretreatment session and the first treatment session were compared using a t-test of differences.

Neurobehavioral Development. The methods and results of behavioral teratology evaluation of trichloromethane (TCM) have been fully reported elsewhere (*8*). The offspring of mice treated throughout gestation and lactation were assessed for possible behavioral effects. A battery of tests of neurobehavioral development, which included measures of the righting reflex, the forelimb placing response, forepaw grasping, the rooting reflex, cliff drop aversion, auditory startle and bar-holding ability were assessed daily from birth through day 15. The screen test as described above was carried out on day 17 and passive avoidance learning on days 22 and 23.

Taste Aversion. Taste aversions produced by TCM in adult mice have been fully described elsewhere (*9*). Individually housed mice were gradually adapted to 30 min per day access to water. After fluid consumption stabilized they were given access to a solution of 0.3% sodium saccharin during the 30-min drinking period. Immediately following the saccharin drinking session the subjects were gavaged with a dose of TCM or vehicle (N = 10 per group). For 10 subsequent days, the subjects were given a two bottle preference test between saccharin and water and following each test the appropriate treatment was administered. Illness-inducing effects are seen as a conditioned aversion to the saccharin solution with which the treatment has been paired. Data are expressed as percent saccharin consumed during the preference tests.

Treatments

The four trihalomethanes with their code, drinking water concentration on which some of the doses were chosen, and estimated average daily human intake (ADI) are presented in Table 1. Both acute effects and the effects of varying durations of repeated administration were assessed. Doses for the 14 and 90-day and behavioral teratology study were chosen to represent 300 and 3000 times the estimated ADI. Higher doses were chosen for other studies in an attempt to determine minimally active dosage regimens.

The trihalomethanes were administered by gavage in suspension using a vehicle of 1:8 Emulphor:water. Emulphor (EL-620, GAF Corp., NY, NY) is a polyoxyethylated oil nonionic surfactant useful for suspending water-insoluble agents for administration to laboratory animals (*10*). We have found that large volume gavage can affect mouse behavior when administered shortly before testing. Thus, for studies in which testing was carried out within a

Table 1. Trihalomethanes studied for behavioral toxicity.

Compound	Abbreviation	Highest concentration, μg/l.[a]	Average daily intake μg/kg/day[b]	Lowest doses studied, mg/kg/day[c]
Trichloromethane	TCM	311	10.4	3.1 and 31.1
Dichlorobromomethane	DCBM	116	3.9	1.2 and 11.6
Dibromochloromethane	DBCM	100	3.3	1.0 and 10.0
Tribromomethane	TBM	92	3.1	0.9 and 9.2

[a]Highest concentration reported in finished drinking water (*1*).
[b]Estimated average human daily intake based upon highest concentrations reported in drinking water.
[c]Doses used for 14 and 90 day studies.

few hours of dosing (operant behavior, acute dose-effect) a volume of 5 ml/kg was used. For long-term studies the injection volume was 10 ml/kg. Fresh suspensions were prepared daily. For all repeated dosing experiments the subjects were gavaged daily, 7 days per week, for the duration of the exposure. For some of the studies, a noninjected control group was included.

Acute Effects. Acute dose-effect curves were obtained for all four trihalomethanes on the screen test. Tests were conducted 30, 60 and 90 min after intubation. Five or six doses of each compound were tested (N = 6 per dose). ED_{50} values were estimated by a computer approximation (*11*) to the method of Bliss (*12*).

The conditioned taste aversion evaluation could also be considered a measure of the acute effects of TCM since the effects appeared after the first of 10 doses. Oral doses of 3, 10 and 30 mg/kg as well as a vehicle control were assessed.

14-Day Administration. Two dose groups of each trihalomethane (300 and 3000 times the estimated ADI; Table 1), as well as vehicle and noninjected control groups (N = 8 per group), were treated for 14 days. Swimming behavior was assessed 24 hr following the last treatment.

90-Day Administration. Two doses of each trihalomethane (300 and 3000 times the estimated ADI; Table 1), as well as separate concurrently treated vehicle and noninjected control groups, were exposed for 90 days. Generally eight subjects were included in each group; however, the vehicle control group for DBCM contained six subjects, the high dose DCBM group contained seven subjects, and both DBCM groups contained 11 subjects. Behavioral testing was carried out over 2 days beginning 24 hr after the last treatment. Subjects were evaluated on the screen test, the hole board and the cling test.

30-Day Administration of 100 mg/kg. The four trihalomethanes were administered at 100 mg/kg/day for 30 days. A concurrent vehicle control group was also tested with each compound. Both treatment and control groups consisted of 16 subjects. Passive-avoidance learning was evaluated beginning 24 hr following the final treatment.

60-Day Administration of 100 and 400 mg/kg. Groups of 6-13 mice trained on a DRL 10 sec schedule of milk presentation were given oral treatments 30 min prior to daily behavioral testing for 60 days. Two dose groups of each trihalomethane (100 and 400 mg/kg) were tested as well as one vehicle control group.

Perinatal Administration. Male and female mice were gavaged with vehicle or 31.1 mg/kg/day TCM for 21 days prior to mating, throughout mating (21 days or until a vaginal plug was detected), and the dam was continued with daily gavage throughout gestation and lactation. The pups were also gavaged with the same dose beginning on day 7. Five TCM and five control litters were evaluated for behavioral teratology. Additional details on the methods for this study can be read elsewhere (*8*).

Results

In general the trihalomethanes were without effects in most of the behavioral tests under most exposure conditions. TCM was investigated most thoroughly including behavioral teratology evaluation as well as for ability to produce a taste aversion. It was active at 100 and 400 mg/kg/day in disrupting operant behavior; however, tolerance developed to this effect. A single oral dose of 30 mg/kg resulted in a clear taste aversion and tolerance did not develop to this effect over 10 days administration. TCM did not result in behavioral teratological effects at 31.1 mg/kg/day, nor was it active at 3.1 or 31.1 mg/kg/day for 14 days on swimming behavior or for 90 days on three other behavioral tests.

The other trihalomethanes were not tested for ability to produce taste aversions or for behavioral teratology. They were all active at very high doses of 100 and/or 400 mg/kg on operant behavior, but were inactive at 100 mg/kg/day for 30 days on avoidance learning. They were also inactive at doses of 300 and 3000 times the ADI for 14 days on swimming behavior and were generally inactive when given for 90 days on three other tests. Occasional significant differences between treatment and control groups were observed; but caution should be used in interpreting these since a pattern of effects did not emerge nor were greater effects seen with higher doses, and many separate statistical analyses were performed.

Since many of the results were negative, all of the data for these studies will not be presented. Rather, selected data will be shown to illustrate the conclusions stated above.

Acute Dose Effect

The acute effects of the trihalomethanes were evaluated on the screen test at 30, 60 and 90 min after oral administration. Effects were seen by 30 min with little evidence of much change by 90 min. Table 2 presents the ED_{50} values for each of the trihalomethanes at their most active time point. All four compounds were roughly equipotent. The effects observed at high doses were ataxia and incoordination and, at the highest doses, anesthesia.

Table 2. Potency estimates of acute oral trihalomethane administration on the screen test in mice.

Compound	ED_{50}, mg/kg[a]	95% confidence limits (mg/kg)	Slope[b]
TCM	484	243 – 965	–2.6
DCBM	524	273 – 1007	–2.9
DBCM	454	262 – 788	–2.0
TBM	431	238 – 788	–2.6

[a]Dose estimated to affect 50% of the subjects at time of peak effect. Calculated by the probit method of Baird and Balster (*11*).

[b]In log dose-probit units.

Repeated Administration

14-Day Administration. Groups of subjects treated with two doses of each trihalomethane were tested for swimming endurance. The mean latency to sink for each treatment group is provided in Table 3. Analysis of variance revealed an overall significant effect of treatments [$F(9,70) = 2.33$, $p < 0.05$]; however, using Dunnett's test to compare each group to the noninjected or vehicle control revealed no one group to be significantly affected. The largest effects were increases in endurance by the low dose TCM group and the high dose TBM group. This increased endurance by the high dose TBM group further suggests that the nonsignificant decrease in endurance in the low dose TBM group was not a reliable treatment effect.

90-Day Administration. The same doses that were used in the 14-day study were used for a 90-day subchronic study. Three behavioral measures, the cling test, screen test and hole board, were used. Both doses of each trihalomethane were tested simultaneously with vehicle and noninjected controls. For the cling test and the screen test, the data for the control groups for each trihalomethane were pooled to provide a better estimate of control behavior. This was done primarily because of the relatively large within-group variability in the case of the cling test and the poor power for detecting effects for the screen test due to the nominal form of the data. This pooling resulted in 27 vehicle control subjects and 28 noninjected controls. One-way analysis of variance on the latency to fall in the cling test revealed no significant F-values in comparing treatment to the pooled control groups. The results of the chi-square tests comparing the effects of the various treatments to the pooled control data (vehicle + noninjected) for the screen test are shown in Table 4. Forty-eight separate analyses were performed. There were no effects of either dose of TCM at any of the time points. Four of the 12 analyses for DCBM revealed significant differences. In all four cases, the differences were observed in the low dose group but not in the high dose group. Two of the 12 analyses for DBCM reached statistical significance. As with DCBM, effects on the measure were observed only with the low dose, and DBCM had no effects on the other behavioral measures at this dose. TBM had no significant effect at either dose at any of the time points measured.

The effects of the 90-day treatments on the hole-board test are presented in Table 5. Two-way analyses of variance were performed on these results for each study. No significant effects of treatments nor treatments by objects interaction was observed in any of the studies. In the DBCM and TBM study the subjects explored holes with objects significantly more frequently than holes with no objects, but the object variable was not significant in either the TCM or DCBM study.

30-Day Administration of 100 mg/kg. Figure 1 presents the results of 30-day administration of the trihalomethanes on latency to enter the dark compartment in the passive avoidance learning test. On the training trial prior to shock delivery, subjects rapidly moved from the light to the dark compart-

Table 3. Effects of 14-day oral administration of trihalomethanes on swimming endurance.

Treatment group[a]	Trihalomethane dose, mg/kg/day	Mean latency to sink, sec	SEM
Noninjected control	–	79.7	19.8
Vehicle control	–	75.5	16.2
TCM	3.1	114.7	10.2
TCM	31.1	89.6	16.9
DCBM	1.2	92.2	15.5
DCBM	11.6	80.5	13.6
DBCM	1.0	65.6	9.2
DBCM	10.0	68.9	6.9
TBM	0.9	49.1	5.1
TBM	9.7	119.4	19.1

[a]$N = 8$ mice per group.

Table 4. Effects of 90-day oral administration of trihalomethanes on the screen test.

Treatment	Trihalomethane dose, mg/kg/day	N	Trial 1[a]			Trial 2[a]		
			15 sec	30 sec	60 sec	15 sec	30 sec	60 sec
TCM	3.1	10	NE	NE	NE	NE	NE	NE
TCM	31.1	11	NE	NE	NE	NE	NE	NE
DCBM	1.2	8	NE	S	S	S	S	NE
DCBM	11.6	7	NE	NE	NE	NE	NE	NE
DBCM	1.0	11	NE	S	NE	S	NE	NE
DBCM	10.0	11	NE	NE	NE	NE	NE	NE
TBM	0.9	8	NE	NE	NE	NE	NE	NE
TBM	9.2	8	NE	NE	NE	NE	NE	NE

[a]Effects at stated times sec. during trial. Trials 1 and 2 were separated by 30 min. NE = no effect; S = significantly worse than pooled control subjects by chi-square, $p < 0.05$.

Table 5. Effects of 90-day oral administration of trihalomethanes on the hole-board test.

Treatment group[a]	N	Object present		No object	
		Mean head dips	SEM	Mean head dips	SEM
I. TCM Study					
Noninjected	8	16.5	3.4	15.4	1.8
Vehicle	8	19.0	2.9	19.9	2.7
TCM, 3.1	8	21.7	3.1	21.1	3.8
TCM, 31.1	8	19.0	2.6	15.4	1.9
II. DCBM Study					
Noninjected	8	15.5	2.8	17.2	1.5
Vehicle	6	15.0	2.2	14.7	2.7
TCM, 1.2	8	15.9	1.8	15.4	1.8
TCM, 11.6	7	20.0	1.4	18.7	2.5
III. DBCM Study					
Noninjected[b]	8	15.0	1.9	13.8	3.0
Vehicle[b]	8	19.4	3.2	13.5	2.1
TCM, 1.0	11	13.0	1.9	14.4	2.6
TCM, 10.0	11	16.4	3.0	15.0	1.9
IV. TBM Study					
Noninjected[b]	8	15.0	1.9	13.8	3.0
Vehicle[b]	8	19.4	3.2	13.5	2.1
TBM, 0.9	8	19.1	2.4	16.2	1.7
TBM, 9.2	8	14.2	1.6	12.1	1.2

[a]Dose in mg/kg/day.
[b]The DBCM and TBM studies were conducted concurrently, thus the same control groups were used.

ment with an average latency of less than 10 sec. On the two retention trials 1 and 24 hr later, the latency was markedly increased indicating that the subjects learned the avoidance. Two-way analyses of variance failed to reveal any significant treatment or treatment by trial interaction. In all four studies there was a highly significant test period effect, i.e., the latencies increased from the training to the post-shock trials. Thus, 100 mg/kg/day for 30 days of each of these trihalomethanes had no effect on passive-avoidance learning or initial step-through latency on this test.

60-Day Administration of 100 and 400 mg/kg. Figure 2 shows the mean daily response rates for the vehicle control group throughout the 60 days of treatment plus the three pre- and post-treatment days. Seven subjects began this regimen. One died from a faulty injection on day 5; thus six subjects completed the study. As can be seen in Figure 2, response rates remained quite stable over the course of the study and the variability decreased as all of the subjects' performance in the task improved. Rates of reinforcement (not shown) rose steadily from 2.71 reinforcements/min on day 1 to 3.3 reinforcements/min on day 60.

Figure 3 presents the effects of 400 mg/kg/day TCM on response rates. These results were typical of the effects of active doses of the other trihalomethanes as well. Thirteen subjects began this treatment; however, six died by day 25, thus only

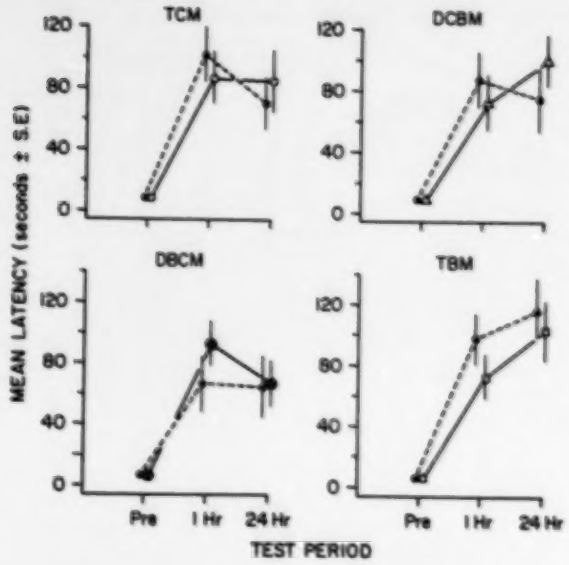

FIGURE 1. Effects of 100 mg/kg/day of (———) trichloromethane (TCM), dichlorobromethane (DCBM), dibromochloromethane (DBCM) and tribromomethane (TBM) or (---) vehicle following 30 days of oral administration on passive-avoidance learning in mice.

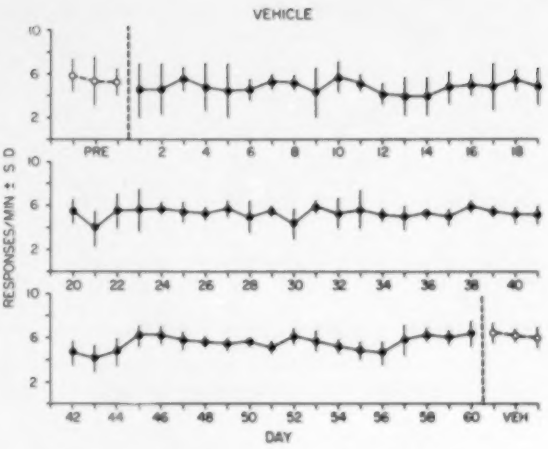

FIGURE 2. Effects of 60 days of an Emulphor:water vehicle on response rates of mice trained on a differential reinforcement of low rate 10 sec schedule of milk presentation. The three open circles prior to and following the 60 days also represent effects of vehicle administration. Treatments were administered by gavage 30 min prior to 30-min experimental sessions.

seven completed this treatment regimen. Animals commonly died during these high dose regimens. Typically their schedule-controlled behavior was unchanged until a few days before they died. It can be seen from the figure that there was an initial large effect of 400 mg/kg given 30 min prior to the session. Response rates and reinforcement rates (not shown) were both substantially decreased. However, rather than a progressive deterioration of performance over the 60-day regimen, some tolerance developed to the initial effects. Variability also decreased over the 60 days although never to the extent seen in the control subjects (Fig. 2). There was no significant effect on the first day following discontinuation of TCM treatment.

This general pattern of effects on the first day of treatment with partial tolerance development was seen with all active doses of the trihalomethanes. Table 6 compares response rates and reinforcement rates on the last pretreatment day and the first treatment day in all nine groups. Significant decreases in response rate, usually accompanied by significant changes in reinforcement rates, were seen with all trihalomethane groups except the 100 mg/kg DBCM group.

Therefore, operant behavior was affected by 100 mg/kg/day of TCM, DCBM and TBM and by 400

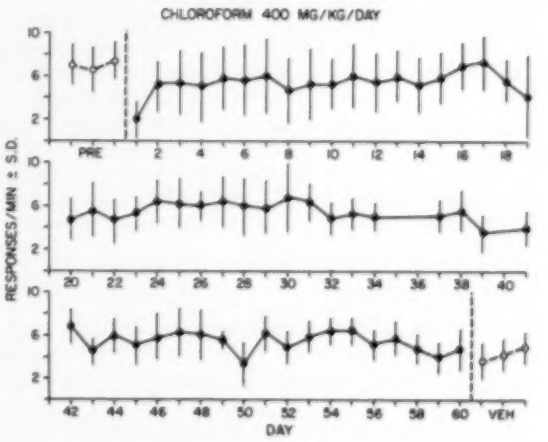

FIGURE 3. Effects of 60 days of 400 mg/kg/day trichloromethane on response rates of mice trained on a differential reinforcement of low rate 10 sec schedule of milk presentation. Details the same as in Figure 2.

mg/kg/day of all four trihalomethanes. Greatest effects were observed early in the regimen with no evidence for progressive behavioral deterioration; in fact, partial tolerance occurred to the effects of these contaminants.

Table 6. Effects of the first day of oral trihalomethane administration on schedule-controlled behavior.

Treatment group[a]	N	Responses per minute		Reinforcements per minute	
		Last pretreatment day (mean ± SEM)	First treatment day (mean ± SEM)	Last pretreatment day (mean ± SEM)	First treatment day (mean ± SEM)
Vehicle	7	4.89 ± 0.91	4.89 ± 0.95	2.93 ± 0.57	2.71 ± 1.07
TCM, 100	12	8.60 ± 0.51	6.38 ± 0.53[b]	2.06 ± 0.70	2.67 ± 0.79[b]
TCM, 400	13	7.40 ± 0.46	2.05 ± 0.53[b]	2.58 ± 0.28	1.23 ± 0.29[b]
DCBM, 100	8	7.20 ± 0.61	4.97 ± 0.76[c]	2.55 ± 0.16	2.54 ± 0.14
DCBM, 400	11	6.84 ± 0.35	2.80 ± 0.56[b]	2.99 ± 0.13	1.39 ± 0.23[b]
DBCM, 100	7	5.85 ± 1.01	5.29 ± 1.12	2.93 ± 0.34	2.50 ± 0.28
DBCM, 400	12	6.94 ± 0.61	1.74 ± 0.58[b]	2.98 ± 0.21	0.96 ± 0.26[b]
TBM, 100	8	5.95 ± 0.14	4.97 ± 0.42[c]	3.22 ± 0.15	3.23 ± 0.10
TBM, 400	6	5.95 ± 0.27	2.86 ± 1.28[c]	3.23 ± 0.23	1.62 ± 0.75[c]

[a]Dose in mg/kg/day.
[b]$p < 0.01$, paired t-test between last pretreatment day and first treatment day.
[c]$p < 0.05$, paired t-test between last pretreatment day and first treatment day.

Perinatal Administration

Only the effects of perinatal administration of TCM were evaluated. These results have been reported elsewhere (*8*). Repeated dosing of the parents through gestation and lactation and the pups from 7 days of age until weaning with 31.1 mg/kg/day had no consistent effect on neurobehavioral development nor on motor performance or passive avoidance learning. The only significant difference between the TCM and vehicle groups was in forelimb placement on days 5 and 7. Since many separate analyses were performed and no differences were seen in other measures of development of function of the extremities (forepaw grasping, bar holding), we did not place much confidence in the reliability of this TCM effect. Thus, there is little evidence of behavioral teratological effects of TCM at 31.1 mg/kg/day.

Taste Aversion

Taste aversions produced by TCM have been reported elsewhere (*9*). Oral doses of 3 and 10 mg/kg/day were without effect after the first day and an aversion to the saccharin did not appear with 10 days of treatment. A significant saccharin aversion was produced after a single pairing with 30 mg/kg TCM and over 10 days of repeated pairings the aversion increased to the point that almost no saccharin was consumed during the two-bottle choice test.

Discussion

A summary of the results of behavioral toxicity evaluation of trihalomethanes is presented in Table 7. TCM has been evaluated most extensively. Acutely, TCM is not very active on most behavioral tests. Its ED_{50} on a simple test of motor performance probably measuring the CNS depressant properties of high doses was 484 mg/kg. This dose is about one-half the LD_{50} obtained under similar conditions (*13*). On the more sensitive measures of schedule-controlled behavior, significant effects were seen at 100 mg/kg. These effects occurred with the first dose and, rather than progressive deterioration of behavior with repeated doses, there was some evidence for tolerance development. Since doses lower than 100 mg/kg were not tested with operant behavior, the minimal active dose cannot be determined, but based upon the magnitude of the effects seen we would not expect doses much lower than 100 mg/kg to have had an effect. The most sensitive measures of the acute effects of TCM was the taste aversion paradigm (*9*). Oral doses of 3 and 10 mg/kg were inactive but 30 mg/kg produced a clear effect. Although taste aversions are a behavioral measure, this does not mean that the toxic effect producing this aversion is mediated by the central nervous system. Taste aversions can be produced by many types of illness-causing agents (*14*). We do not believe that this effect of 30 mg/kg TCM was due to a localized gastric irritation, however, since an even lower dose could produce the aversion by the IP route.

The remainder of the behavioral evaluation of TCM was designed explicitly not to measure acute effects since testing was not carried out until 24 hr following the last treatment. In these studies we were looking for evidence of relatively permanent behavioral changes. A variety of measures and dosage regimens were used, but even up to 100 mg/kg/day for 30 days there was no evidence for behavioral toxicity. The measures used (screen test, cling test, hole-board, swimming and passive-

Table 7. Summary of behavioral toxicity evaluation of trihalomethanes.

	Effect[a]			
	TCM	DCBM	DBCM	TBM
Acute ED_{50}, mg/kg	484	524	454	431
14-day, swimming				
300 times ADI/day	No	No	No	No
3000 times ADI/day	No	No	No	No
90-day, cling, screen and hole-board				
300 times ADI/day	No	No[b]	No[c]	No
3000 times ADI/day	No	No	No	No
30-day 100 mg/kg, learning	No	No	No	No
60-day, operant behavior				
100 mg/kg/day	Yes	Yes	No	Yes
400 mg/kg/day	Yes	Yes	Yes	Yes
Perinatal administration				
31.1 mg/kg/day	No	NT	NT	NT
Taste aversion				
3 mg/kg/day	No	NT	NT	NT
10 mg/kg/day	No	NT	NT	NT
30 mg/kg/day	Yes	NT	NT	NT

[a]No = no effect; yes = significant effect; NT = not tested.
[b]Four of 12 screen tests were significant, but no clear pattern suggesting behavioral toxicity.
[c]Two of 12 screen tests were significant, but no clear patterns suggesting behavioral toxicity.

avoidance learning) were not selected to be particularly sensitive, but rather as initial screening tools. Had effects at these high doses been found, lower doses would have been tested in more sensitive measures (e.g., operant behavior). In addition, effects were not seen as a result of perinatal administration of 31.1 mg/kg/day. This study can be considered an initial screening of the behavioral toxicity associated with long-term oral TCM exposure. There is no evidence from these results with doses 300 and 3000 times or more the ADI of TCM from contaminated drinking water that progressive neurobehavioral toxicity is likely to be a problem.

The other trihalomethanes were not evaluated quite as extensively as TCM, but what data there is suggests that they, too, are not very active behaviorally (Table 7). The ED_{50} values of DCBM, DBCM and TBM were comparable to those for TCM. With the exception of DBCM they all had effects at 100 mg/kg on operant behavior. All the trihalomethanes had pronounced effects on operant behavior at 400 mg/kg/day. Like these effects of TCM, effects on operant behavior were greater early in the 60-day treatment regimen; thus they were not progressive. Fourteen and 90-day studies of 300 and 3000 times the ADI of DCBM, DBCM and TBM were without consistent behavioral effects. Occasional significant differences were observed between treatment and control groups but these should be interpreted with caution. For example, 90-day administration of the low dose of DCBM (1.2 mg/kg/day) had a significant effect on the screen test of motor coordination on 4 of the 12 analyses performed. In assessing the implications of this, it should be kept in mind that: (1) with a large number of statistical analyses performed some will be significant by chance; (2) these four differences are not independent observations, since once a subject has fallen from the screen by 30 sec (as six did at the low dose on trial 1), they will also be counted as fallen at 60 sec; (3) the effects were only seen with the low dose; (4) no effect of DCBM was seen in the cling test or hole-board tested in these same subjects. Nevertheless, it is possible that DCBM may have some neurobehavioral effects that were not dose-dependent and which may need to be evaluated by further research. However, even 30-day treatment with 100 mg/kg/day of any of the trihalomethanes had no effects on any of the measures obtained from a passive avoidance learning task. We have not evaluated DCBM, DBCM or TBM for behavioral teratology and they have not been tested in the taste aversion paradigm. The latter would be most interesting to do since it was most sensitive to the effects of TCM.

The evaluation of the behavioral toxicity of trihalomethanes represented our first efforts in the assessment of the safety of drinking water contaminants. As a result of this experience, we have made a number of changes in the behavioral battery as well as in the choice of dosage regimens (*15,16*). Subsequent studies have not based the choice of doses on ADI's estimated from reports of levels of drinking water contamination. Rather, an attempt is now made to determine maximal no-effect levels. This is done by choosing doses for repeated exposure based upon the acute behavioral and lethal effects. Doses

for 14-day studies are chosen as 1/10 and 1/100 of the LD_{50} for the chemical. If no adverse behavioral effects are observed in the subacute study, then the subchronic study is executed at the same doses. In addition, where feasible, the subchronic studies are carried out by exposing subjects via their drinking water rather than by daily gavage. If this approach to dosage choices had been used with the trihalomethanes, then the 14- and 90-day studies would have been carried out at substantially higher doses. The use of higher doses would have allowed us to make a better estimate of minimal effective dosage regimens for behavioral effects.

We have also made changes in the dependent measures as a consequence of our experience with the trihalomethanes (*15,16*). A number of new measures have been added. They include photocell motor activity, body weight, food intake, a standardized behavioral observation, body temperature measurement, sensory tests (olfaction and nociception), social behavior, and taste aversion testing. In addition, unlike the case for the trihalomethanes where most testing was initiated one day after the last dose of the regimen, many of the assessments are now made throughout the exposure. We no longer use the hold-board test since we believe that photocell motor activity is assessing some of the same behavioral functions. The screen test has been modified to record latency to climb to the top of the screen rather than the nominal result of success or failure. On the other hand, few changes have been made in our approach to behavioral teratology assessment. Operant behavioral testing has been deemphasized since it lends itself best to within-subjects designs and in a screening program the large effort required may not be justified. We still find it useful to characterize maximal no effect levels by a procedure involving an ascending dosage series which continues until effects are found.

In conclusion, we have presented our evaluation of the behavioral toxicity of trihalomethane contaminants of drinking water. The purpose of these studies was twofold: to develop an approach to safety evaluation of drinking water contaminants and to provide some data on the behavioral effects of trihalomethanes. The trihalomethanes have not proven to be very active on most behavioral tests and thus regulatory decisions for these compounds are unlikely to need to focus on behavioral toxicity. Our results with the trihalomethanes, however, have been very helpful in the development of a strategy for assessment of the behavioral toxicity of drinking water contaminants.

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